IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

| | | |
|---|---|---|
| Applicant(s) | : | Alexandre MARTI et al. |
| Serial No. | : | 09/673,~~817~~ 871 |
| Filed | : | April 22, 1999 |
| Title | : | SOLUTION FOR DIAGNOSING OR TREATING TISSUE PATHOLOGIES |
| Art Unit | : | 1617 |
| Examiner | : | S. J. Sharareh |

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

**DECLARATION UNDER 37 C.F.R. § 1.132**

SIR:

I, GEORGES WAGNIÈRES, Ph. D., declare as follows:

1. I am a joint inventor of the subject matter of the above-identified application.

2. I received a doctorate degree from the Swiss Federal Institute of Technology (EPFL) in 1992. From July 1993 to August 1994, I was a postdoctoral fellow at the Harvard Medical School, Wellman Laboratories of Photomedicine, Boston Massachusetts.

3. I am currently an Adjoint scientifique (Project Leader) at the Swiss Federal Institute of Technology (EPFL) in Lausanne, Switzerland, and have served in this capacity since September 1994. From May 1992 to June 1993, I also served in the same capacity as I currently do at the EPFL.

4. In the past fourteen years, I have been a co-author of at least 69 papers published in peer-reviewed journals and have been an author or coauthor of 6 books, as well as over 60 other publications. Many of these publications are in the area of photodynamic therapy and diagnosis. (*See, e.g.*, F. Ludicke et al. "Photodynamic diagnosis of ovarian cancer using aminolevulinic acid hexylester: A preclinical study", Brit. J. of Cancer, 88(11), pp 1780-

1784, 2003; Radu A. et al. "Photodynamic therapy of early squamous cell cancers of the esophagus", Gastrointestinal Endoscopy Clin. North America, 10(3), pp 439-460, 2000).

5. I am a member of a number of professional societies, including the International Society for Optical Engineering, the Optical Society of America and the European Optical Society. I have served on the editorial board of a journal specializing in diagnostic optics, The Journal of Biomedical Optics, and have chaired numerous international conferences. Additional facts about my background and qualifications including a list of my publications are set forth in my *curriculum vitae*, attached as **Exhibit A**. An updated list of published articles is attached at the back of my *curriculum vitae.*

6. I have read and understand the outstanding Office Action mailed July 25, 2003 and the cited U.S. Patent No. 6,034,267 (hereinafter the "'267 patent"), issued on March 7, 2000 to Gierskcky et al.

7. The present application provides a solution for administration to a patient for diagnosis or treatment comprising a physiologically acceptable solvent and an ester of 5-aminolevulinic acid (E-ALA) which is present in the solution at a concentration of less than 1% by weight.

8. My co-inventors and I, and/or those working under our direction and supervision, performed experiments described in the application and obtained the results discussed therein. Below I provide additional details on these results, as well as subsequent studies.

9. Protoporphyrin IX ("PpIX"), a heme precursor, is used as a fluorescence marker and photosensitizing agent in photodynamic therapy. PpIX forms and accumulates in tissues with a high cellular turnover, *e.g.*, tumors. Therefore, an increase in intracellularly generated PpIX formation in response to exogenous stimulation by administration of 5-aminolevulinic acid (ALA) may be used for tumor destruction by photodynamic (PDT) therapy. ALA-mediated photodynamic therapy (PDT) was an emerging field for treatment of cancers since about 1989-1990. However, since ALA-mediated PDT is limited by ALA's poor ability to diffuse through cell membranes, solutions containing high doses of ALA of about 180 mM (about 3% w/w) have to be administered to increase PpIX production in the deep layers of cancerous lesions. Therefore, I have studied the comparative effects of administration ALA-esters and of ALA to determine optimal concentrations of these PpIX precursors for use in PDT and photodiagnosis.

10. In one study, two cell lines derived from human transitional cell carcinoma of the bladder, J82 and T24 cells, respectively, were incubated with ALA or esters of ALA, as described in P. Uehlinger et al. "5-Aminolevulinic acid and its derivatives: physical chemical properties and protoporphyrin IX formation in cultured cells", J. Photochem. Photobiol. B: Biol., 54, pp 72-80, 2000 (**Exhibit B**). The esters of ALA studied included ALA-methylester, ALA-ethylester, ALA-butylester, ALA-hexylester, and ALA-octylester.

11. In this study, I found that ALA-butylester, ALA-hexylester, and ALA-octylester not only produced PpIX formation at much lower concentrations than ALA, but also produced higher amounts of PpIX, as shown in the graphs for the J82 and T24 cells, respectively, which are adapted from Figure 3 of Uehlinger et al. 2000, and are attached as **Exhibit C**.

12. Experiments were also performed on bladder tissue *in vitro*, in which PpIX formation after administration of ALA or ALA-ethylester, ALA-butylester, ALA-hexylester, and ALA-octylester, were measured, as described in A. Marti et al. "Optimization of the Formation and Distribution of Protoporphyrin IX in the Urothelium: an In Vitro Approach", J. Urology, 162(2), pp 546-555, 1999, attached as **Exhibit D**. The results again demonstrated higher amounts of PpIX formation at much lower concentrations of ALA-ethylester, ALA-butylester, ALA-hexylester, and ALA-octylester than with ALA. These results are shown in **Exhibit E**, adapted from Figure 4 of Marti et al. 1999. A Table summarizing these results, attached as **Exhibit F**, shows the optimal concentrations of ALA and ALA esters for PpIX production. The Table (**Exhibit F**) indicates that the ALA-butylester, ALA-hexylester and ALA-octylester all achieved optimal PpIX levels at concentrations of less than 1%. The data in **Exhibits E** and **F** demonstrate that various concentrations below 1% of ALA-ethylester, ALA-butylester, ALA-hexylester, and ALA-octylester achieve high levels of PpIX formation.

13. *In vivo* clinical studies were performed to compare induction of PpIX with 8 mM of ALA-hexylester and 180 mM of ALA in a human pTa G2 cancer patient, as described in Lange et al. "Photodetection of early human bladder cancer based on the fluorescence of 5-aminolaevulinic acid hexylester-induced protoporphyrin IX: a pilot study, Br. J. Cancer, 80(1/2), pp 185-193, 1999 (**Exhibit G**). For ALA-HCl, 180 mM corresponds to 30.17 mg/ml or 3.02% (w/w), whereas 8 mM of ALA-hexylester HCl corresponds to 2 mg/ml or 0.2% (w/w). The results, derived from Lange et al, p. 190, last paragraph, are shown in **Exhibit H**. They demonstrate that higher levels of PpIX were formed with much lower concentrations of ALA-hexylester than ALA.

14. Prior to April 1998, other researchers studied the effects of concentrations of ALA-esters that were about two orders of magnitude higher than the concentrations of the present invention. For example, Peng et al. "Build-up of esterified aminolevulinic-acid-derivative-induced porphyrin fluorescence in normal mouse skin" J. Photochem. Photobiol B: 34 (1996):95-96 (**Exhibit I**), administered methylester, ethylester and propylester of ALA in a concentration of 150 mg/kg, *i.e.*, about 20% (w/w). Our studies, based on a mean patient weight of 75 kg, administered a dose of approximately 1.3 mg/kg, which is about two orders of magnitude lower than that used in Peng et al.

15. Therefore, it was unexpected by me that the lower doses of ALA-esters would produce higher levels of PpIX than the lowest doses of ALA-esters studied at the time of the present invention.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the patent or any reexamination certificate issued therefor.

Dated: 26th of JANUARY 2004

G. Wagnières

GEORGES WAGNIÈRES, Ph. D.